Ticks and Tick-borne Diseases 13 (2022) 101966

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Plasticity in the timing of detachment of an Eurasian-African songbird tick, *Ixodes frontalis*

D.J.A. Heylen [a,b,*], A.R. Van Oosten [c], G. Fracasso [a,c], E. Matthysen [c]

[a] Eco-Epidemiology Group, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
[b] Interuniversity Institute for Biostatistics and Statistical Bioinformatics, Hasselt University, Diepenbeek, Belgium
[c] Evolutionary Ecology Group, Department of Biology, University of Antwerp, Wilrijk, Belgium

ARTICLE INFO

Keywords:
Plasticity
Ixodes frontalis
Ixodes arboricola
Ixodes ricinus
Dispersal
Transmission

ABSTRACT

In non-permanent parasites, host detachment should take place in an environment that ensures the continuation of their life cycle. Timing of detachment - in combination with the host's space use - affects dispersal and transmission success of the parasites and of the pathogens they vector. Before reaching the adult reproductive stage, ticks need to go through multiple immature developmental stages (larva and nymph), each feeding on host blood. In between the feeding bouts, they often remain in the off-host environment for considerable periods of time. With this study, we aimed to obtain more insight in *Ixodes frontalis*' off-host habitat use by comparing its detachment pattern in different life stages with that of two habitat-specialized ticks also found on birds: the endophilic tree-hole tick (*Ixodes arboricola*) and the exophilic sheep tick (*Ixodes ricinus*), the latter living in humid understory vegetation of forests. For this, we artificially infested hole-roosting (great tits, *Parus major*) and open-roosting (blackbirds, *Turdus merula*) birds with ticks under laboratory conditions, and recorded whether detachment occurred during the day or the night. We hypothesize that nocturnal detachment improves off-host mating opportunities and host localization, whereas diurnal detachment optimizes tick dispersal. *Ixodes frontalis* nymphs detached during the night, especially when feeding on blackbirds. This behaviour was very similar to that of *I. arboricola* (larva and nymph) feeding on great tits. In contrast, *I. frontalis* larvae detached during the day, especially when feeding on great tits, which resembles that of *I. ricinus'* feeding behaviour (larva and nymph). *Ixodes frontalis* left the host within seven days, immediately after completion of the blood meal. This is similar to both developmental stages of *I. ricinus* but contrasts with the very long (up to 20 days) feeding duration in *I. arboricola*. Thus *I. frontalis* shows strong plasticity, switching from dispersal-centered (larvae) to host-centered (nymphs) detachment behaviour. Findings are discussed with regard to the ticks' habitat use, dispersal, life history and host specificity.

1. Introduction

In parasites with low intrinsic mobility, dispersal often takes place in association with host movements (Dick and Patterson, 2007; McCoy et al., 2003; Poulin, 2007). Therefore, temporal aspects in the life-history of parasites that are linked to host space use heavily affect the probability of transmission, and ultimately the basic reproductive number (R_0). In non-permanent parasites (i.e. parasites spending part of their life separated from the host), off-host survival is crucial for the reproductive success and continuation of the life cycle (Poulin, 2007; Price, 1980). To maximize transmission, mechanisms have evolved that control the departure from the host. Particularly in mobile hosts spanning a range of habitat types, physical and physiological cues linked to the parasite's optimal habitat are assumed to determine its departure.

Ixodid ticks are non-permanent blood-sucking ectoparasites and are characterized by a low intrinsic mobility, hence intrinsic dispersal capacity. Therefore, ticks depend on host movement for transportation to sites where they subsequently develop and contact the next host. Three-host ixodid ticks spend long periods of time off-host. They typically take a single blood meal per life stage lasting several days before detachment and moulting to the next development stage, and thus spend most of their life off-host (Hillyard, 1996). Consequently, each life stage experiences the dual pressures of survival on and off the host. Ticks are vectors for multiple micro-parasites that include the bacterium complex

* Corresponding author at: Kronenburgstraat 25, 2000, Antwerp, Belgium.
E-mail address: dheylen@itg.be (D.J.A. Heylen).

https://doi.org/10.1016/j.ttbdis.2022.101966
Received 1 February 2022; Received in revised form 12 April 2022; Accepted 2 May 2022
Available online 13 May 2022

Borrelia burgdorferi s.l. causing Lyme borreliosis/disease in humans (Ginsberg et al., 2005). Ixodid ticks are frequently found feeding on birds. Birds are very mobile vertebrates, and are recognized to play a pivotal role in the spread of certain zoonotic diseases. They act as long-range carriers for parasites, among which vector-borne diseases to humans, thereby establishing new disease foci far away from existing ones (Chen et al., 2005; Hubalek, 2004; Lanciotti et al., 1999; Ogden et al., 2008). Even when birds are not infected, they can function as vehicles for infected ticks (Heylen et al., 2014b; Heylen et al., 2017b) which may - depending on local abiotic conditions and vertebrate communities – initiate disease foci.

Of the most common ticks found on songbirds in the Holarctic – and frequently observed in Northern Africa as well - *Ixodes frontalis* (Panzer) is likely the most overlooked because of its morphological resemblance to the very abundant *Ixodes ricinus* L. (Heylen et al., 2014a). It infests a broad range of terrestrial birds, including songbirds that breed in the open (e.g. thrushes, Turdidae). It also carries several pathogenic agents, including *B. burgdorferi* s.l. bacteria, '*Candidatus* Neoehrlichia mikurensis' and Chizé virus (Chastel et al., 1999; Doby, 1998; Estrada-Peña et al., 1995; Heylen et al., 2017a; Heylen et al., 2013; Movila et al., 2013; Rubel et al., 2021). Its off-host habitat and host-finding strategy are much under debate, i.e. the places where the tick spends most of its life developing to the next stage, and how and when it attacks hosts. The tick has been linked with bird nests (Hillyard, 1996) of open nesting birds, and hole-breeding birds (pers. obs. Maxime Madder and Joris Elst). However, *I. frontalis* has also been collected by flagging methods on understory vegetation in parks and forests (Bona and Stanko, 2013; Doby, 1998; Gilot et al., 1997; Schorn et al., 2011; Rubel et al., 2021), the habitat that is similar to that of the sheep tick (*Ixodes ricinus*), but also underneath bamboo in France and Northern Italy (Plantard et al., 2021). In all studies, however, the numbers of *I. frontalis* found are surprisingly low, especially given the fact that the reproductive output (i.e. number of larval ticks) is more than 3,000-4,000 larvae per female (D. Heylen, unpublished data from laboratory colony).

In parasites that are dependent on their host for dispersal, timing of detachment is expected to be linked to host activity. In order to unravel whether detachment behaviour of *I. frontalis* has evolved to promote dispersal (i.e. detachment when the host is active and mobile) or host localization and off-host mating opportunities (i.e. detachment when the host is sleeping; often at the same roosting spots), we infested two diurnally active bird species with contrasting ecologies (the hole-roosting *Parus major* L. and the open-roosting *Turdus merula* L.) with *I. frontalis*. Moreover, we infested birds in the morning and evening, to determine if ticks detached after a fixed time interval or preferentially during the day or the night. Detachment during the night increases the likelihood for the tick to end up near the bird's roosting site: inside the tree-hole (great tit) or underneath branches in an open-nesting songbird (blackbird). Detachment during the day-light hours increases the probability to end up scattered at the base of trees, thickets or understory vegetation. Outcomes are compared with detachment patterns of two well-studied habitat-specialized ticks frequently found on songbirds as well (Heylen and Matthysen, 2010) in order to better understand *I. frontalis*' lifestyle: the endophilic tree-hole tick (*Ixodes arboricola* Schulze & Schlottke) and the exophilic sheep tick (*I. ricinus*) living in humid understory vegetation of forests and parks.

2. Materials and methods

2.1. Study design

Birds (great tits, blackbirds) were infested with ticks (larvae, nymphs) either at dawn or at dusk; hereto birds were randomly allocated to the two levels of 'moment of infestation' ('dawn': 7:30 am, or 'dusk': 7:30 pm) and this for each bird × tick × stage-combination (Table 1). Two hypotheses were tested: H1 - duration until detachment is equal for the morning- and evening-infested birds. This means that the time to tick detachment is pre-programmed and independent of the bird' circadian activity. We therefore expect that equal numbers of ticks detach during day (from 7:30 am to 7:30 pm) and night (from 7:30 pm to 7:30 am) for both bird species, and this in both developmental stages. H2 - in exophilic ticks (*I. ricinus* and possibly *I. frontalis*) placed on great tits, all detachments occur during the daylight hours (i.e. outside their tree-holes) irrespective of their moment of infestation. In blackbirds, the detachments are spread over day and night, given that this bird sleeps in the open.

In this analysis, we included a large number of birds (Table 1) that were all infested following a standardized protocol developed over the past 14 years (2008-2022) in the Evolutionary Ecology Lab (University of Antwerp). Given the birds' physiological status may vary seasonally, birds were infested at the same time of the year (autumn: October-November). Additional birds, only used for comparisons of duration until detachment after placement on the birds, were infested in winter or summer (Table 1). Space limitations did not allow to test all bird × tick × stage-combination simultaneously. Great tits were infested with either 25 larvae or 12 nymphs while blackbirds were exposed to either 50 larvae or 20 nymphs (Heylen and Matthysen, 2008; Hudde and Walter,

Table 1
Overview of the birds (*Turdus merula, Parus major, Cyanistes caeruleus, Sitta europaea*) kept in captivity, and the ticks (*Ixodes frontalis, I. ricinus* and *I. arboricola*) placed on them. Birds that were monitored for nocturnal detachments were exposed at two moments (morning and evening) and trays underneath the cages were inspected for detached ticks twice (morning and evening). Additional birds were exposed around noon, and trays were inspected once daily (around noon). Temperature and humidity in aviary and lab conditions co-varied with outdoor climatic conditions.

	I. frontalis	*I. ricinus*	*I. arboricola*	Conditions	Reference
Nocturnal Detachment					
larvae - *P. major*	/8 (30/bird)	12 (30/bird)/	12 (30/bird)/	Lab - Autumn	[1]/unpubl.
nymphs - *P. major*	/8 (12/bird)	11/13 (12/bird)/	12 (12/bird)/	Lab - Autumn	[1] /unpubl.
larvae - *T. merula*	19 (50/bird)	8 (50/bird)		Lab - Autumn	unpubl.
nymphs - *T. merula*	13 (20/bird)	11 (20/bird)		Lab - Autumn	unpubl.
Additional					
larvae - *S. europaea*			3 (30/bird)	Aviary - Winter	unpubl.
larvae - *C. caeruleus*			4 (30/bird)	Aviary - Winter	unpubl.
larvae - *P. major*			5 (30/bird)	Aviary - Winter	unpubl.
nymphs - *P. major*			4/1 (12/bird)	Aviary - Winter	unpubl/[3]
nymphs - *C. caeruleus*		31 (12/bird)		Lab - Summer	[2]
nymphs - *P. major*		28 (17/bird)		Lab - Summer	[2]

[1] Heylen, D.J.A., Matthysen, E., 2010. Contrasting detachment strategies in two congeneric ticks (Ixodidae) parasitizing the same songbird. Parasitology 137, 661-667.
[2] Heylen, D.J.A., Madder, M., Matthysen, E., 2010. Lack of resistance against the tick *Ixodes ricinus* in two related passerine bird species. International Journal for Parasitology 40, 183-191.
[3] White, J., Heylen, D.J.A., Matthysen, E., 2012. Adaptive timing of detachment in a tick parasitizing hole-nesting birds. Parasitology 139, 264-270.

1988; Literak et al., 2007), which are both within the range of natural conditions. After a habituation period of at least 7 days in captivity, birds were artificially infested with ticks at either 08:00 am or 08:00 pm ('moment of infestation'). Using tweezers (nymphs) or brushes (larvae), ticks were put underneath the feathers on the head of the bird, following their natural attachment preference (Fracasso et al., 2019). Immediately afterwards, birds were kept for 1.5 hours in an air-permeable cotton bag inside a darkened cage which kept them inactive (Heylen and Matthysen, 2008).

2.2. Ectoparasite and host

2.2.1. Ticks

I. frontalis larvae were obtained from engorged adult female ticks collected in late winter 2015 from experimentally infested song thrushes (*Turdus philomelos* L.) and from wild redwings (*Turdus iliacus* L.) captured near Antwerp, Belgium. They were kept at 25°C and 90% relative humidity until the emergence of larvae from their eggs. After engorgement, *I. frontalis* larvae were kept individually in tubes at 25°C and 83% relative humidity until development to the nymphal stage. *Ixodes ricinus* larvae were obtained from a German laboratory colony (IS Insect Services GmbH, Berlin). All *I. arboricola* ticks were obtained in the spring of 2008 from nestboxes in which *P. major* nestlings were infested with ticks. *Ixodes ricinus* nymphs were caught (late summer) by dragging a white flannel flag over suitable vegetation. Field-caught nymphs were kept in the same climatic conditions as those of the larvae (see above) for a sufficiently long period of time (> 3 weeks) before placing them on the birds. Until infestation, all unfed tick stages were kept at 12h:12h light: dark photophase (with the exception of the tree-hole tick *I. arboricola*, kept in complete darkness as in its habitat), 20°C:10°C temperature cycle and 83% relative humidity.

2.2.2. Birds

Three sets of birds were used in the manuscript (see Table 1 for an overview): **(a)** Birds kept indoor in the lab (unheated room), exposed at dusk and dawn in autumn/winter and with cage trays checked every 12 hours to register night/day detachments (see 2.3. below). The 26 *Turdus merula* individuals were obtained from a licensed breeder (Houthalen, Belgium) that kept birds inside tick-free aviaries with paved floors, that are free of ticks. The 114 *Parus major* individuals were all captured at two sites near Antwerp (Belgium) with mist nets under licence from the Belgian Ringing Scheme (Brussels). During the experiments, birds were maintained with a photophase beginning at 07:30 am and a scotophase beginning at 07:30 pm. Temperature varied with daily ambient outdoor temperatures. Birds received food and water *ad libitum*. Two additional sets of birds were included, in order to descriptively situate the average detachment profiles of the above-mentioned birds: **(b)** 59 birds kept indoor, but exposed at noon in summer (great and blue tits) and with trays checked every 24 hours; **(c)** 17 birds that were kept in outdoor conditions, exposed around noon (winter), with trays checked every 24 hours. Wild birds were captured with licence (ANB/BL-FF/VERGUN14-00138) of the Agency for Nature and Forests (Flemish Government, Belgium). The tick infestation procedure was approved by the Ethics Committee for Animal Experiments of the University of Antwerp (2009-32 and 2014-49).

2.3. Study of nocturnal detachment and feeding duration

After tick exposure, birds were placed individually in a cage with a wire-mesh floor (40 cm × 80 cm). Below the wire-mesh floor was a removable plastic tray containing damp filter paper and edges streaked with vaseline to prevent ticks from escaping. The engorged ticks that dropped through the mesh cage were collected two times a day: the morning check started at 07:00 am, the evening check around 07:00 pm. Each check took on average 1 to 1.5 hours. Trays were removed, and checked in good light conditions outside the room where all birds were caged. Consequently, the birds were kept in the dark until all trays had been examined. Some immature ticks were presumably lost because they could not be found amongst the faeces and food remains beneath the wire-mesh or because they were eaten by the hosts before detachment. The detachment of engorged ticks was followed up for a maximum period of 20 days after infestation. Attached ticks that remained on the birds were removed with tweezers.

2.4. Statistical analysis

Generalized estimation equations (GEE) were fitted (logit-link, and binomial distributed residuals) to model the proportion of ticks that detached, taking into account the statistical dependence of measurements on the same bird. The proportion of engorged ticks that detached nocturnally was modelled against the moment of infestation, the tick species and their interaction. We used methods of survival analysis (time-to-event data) for modelling the duration until tick detachment (see Cox and Oakes, 2004 for general information). The duration until detachment (in days) was modelled by a marginal cox proportional hazards model for clustered data (Shu and Klein, 1999) with tick species and the moment of infestation added to the model. Those ticks that were removed at the end of the experiment were handled as right-censored data. Data is visually represented by Kaplan-Meier curves. All data analyses were conducted in SAS v 9.4 (SAS Institute, Cary, North Carolina).

3. Results

3.1. Timing of detachments

For each life stage, the findings for the two habitat-specialized ticks (*I. arboricola* and *I. ricinus*) are reported, and compared with *I. frontalis*. An overview of all pair-wise comparisons is provided in Table 2.

3.1.1. Larval detachment in relation to tick and host species

Of the larvae that successfully fed on great tits, the majority of *I. arboricola* detached by night (87 ± 3% per bird), while this happened less often in *I. ricinus* (12 ± 4% per bird; $\text{logit}_{(\text{I.a} - \text{I.r.})} = 3.97 \pm 0.47$; $\chi^2 = 8.48$; df = 1; $P < 0.0001$) and *I. frontalis* (21 ± 8% per bird; $\text{logit}_{(\text{I.a} - \text{I.f.})} = 3.35 \pm 0.52$; $\chi^2 = 6.47$; df = 1; $P < 0.0001$). Nocturnal detachment in *I. ricinus* (35 ± 7% per bird) and *I. frontalis* (45 ± 5% per bird) occurred more often in blackbirds than in great tits ($\text{logit}_{(\text{T.m.} - \text{P.m.})} = 1.32 \pm 0.36$; $\chi^2 = 3.66$; df = 1; $P = 0.0003$).

3.1.2. Larval detachment in relation to moment of exposure

In both bird species there was a significant interaction between 'tick species' and 'moment of exposure' (great tits: $\chi^2 = 6.96$; df = 1; $P = 0.0083$; blackbirds: $\chi^2 = 5.28$; df = 1; $P = 0.021$): *I. ricinus* larvae placed on birds in the morning detached less often at night than the ones placed in the evening ($\Delta_{(\text{dusk-dawn})}$ great tit: 18.6 ± 6.9% per bird; blackbird: 21.7 ± 12.9% per bird). The reverse pattern was observed in *I. frontalis* ($\Delta_{(\text{dusk-dawn})}$ great tit: -22.8 ± 13.5% per bird; blackbird: -28.05 ± 7.8% per bird).

3.1.3. Nymphal detachment in relation to tick and host species

Similar to larvae, differences among tick species were observed in their propensity to detach at night. In great tits, a very high proportion of *I. arboricola* individuals detached nocturnally (90 ± 3% per bird), which was much higher than the proportion in *I. ricinus* (23 ± 5% per bird; $\text{logit}_{(\text{I.a} - \text{I.r.})} = 3.37 \pm 0.42$; $\chi^2 = 8.07$; df = 1; $P < 0.0001$). Also in *I. frontalis*, a considerable proportion of nocturnally detached nymphs was collected (61 ± 10% per bird), but this was still much lower than the proportion in *I. arboricola* ($\text{logit}_{(\text{I.a} - \text{I.r.})} = 3.37 \pm 0.42$; $\chi^2 = 8.07$; df = 1; $P < 0.0001$). In contrast, in blackbirds, *I. frontalis* nocturnal detachments happened more often than in great tits (86 ± 4% per bird).

Table 2
Mean infestation parameters per bird (± SE) of the immature developmental stages of *Ixodes ricinus, Ixodes frontalis* and *Ixodes arboricola* fed on the hole-roosting *Parus major*, and the open-roosting *Turdus merula*.

	Larvae			Nymphs			
	Morning	***Evening***	***C***	***Morning***	***Evening***	***C***	***D***
Night detachments % (N° ticks/N° birds)							
Parus major							
Ixodes ricinus	3±1(100/6)	21±7(105/6)	H,.02 - A	27±8(74/9)	18±7(51/7)	N - A	N
Ixodes frontalis	32±13(101/4)	9±4(106/4)	L,.03 - A	49±18(23/4)	73±8(26/4)	N - A	H,< .01
Ixodes arboricola	85±4(77/6)	89±5(100/6)	N	90±5(48/10)	89±4(65/9)	N	N
	abc	aab		aab	abc		
Turdus merula							
Ixodes ricinus	24±6(120/4)	46±11(122/4)	H,.03 - B	34±11(79/5)	42±14(66/6)	N - A	N
Ixodes frontalis	59±6(924/10)	31±5(517/9)	L,< .001 - B	79±6(95/7)	93±3(74/6)	H,< .001 - B	H,< .001
	ab	ab		ab	ab		
Duration in days							
Parus major							
Ixodes ricinus	3.59±0.04	3.75±0.05	H,< .01 - A	3.89±0.08	4.03±0.05	N - A	N
Ixodes frontalis	5.15±0.05	4.00±0.02	L,< .001 - A	4.43±0.07	4.37±0.11	N - A	N
Ixodes arboricola	12.15±0.6(*24)	8.61±0.60(*18)	L,< .01 - B	7.71±0.71(*2)	7.65±0.58(*8)	N - B	L,<0.01
	abc	abc		abc	abc		
Turdus merula							
Ixodes ricinus	3.24±0.03	3.16±0.05	N - B	3.09±0.04	3.03±0.09	N - B	L,< .001
Ixodes frontalis	4.46±0.02	4.75±0.02	H,< .05 - A	3.72±0.04	3.53±0.05	L,< .05 - B	L,< .001
	ab	ab		ab	ab		
Infestation success %							
Parus major							
Ixodes ricinus	28±8	29±6	N - A	35±7	31±7	N - A	N
Ixodes frontalis	42±10	44±14	N - A	36±9	41±8	N - A	N
Ixodes arboricola	21±5	28±7	N	23±5	32±6	N	
	abc	aaa		aab	aba		
Turdus merula							
Ixodes ricinus	30±7	31±6	N - A	40±8	27±6	N - A	N
Ixodes frontalis	36±4	35±4	N - A	34±7	31±9	N - A	N
	aa	aa		aa	aa		

Night detachment: % of all successfully fed ticks that detached during the night;
Feeding duration: Time between exposure and detachment in days;
Infestation success: % of all ticks that were placed on the birds and successfully fed
In column 'C': Left-to-right comparisons (i.e. Morning vs Evening exposed), evening exposed significantly lower ('L', P-value) or higher ('H', P-value) than morning exposed birds, 'N': no difference;
In column 'C': '- A', '- B' describe host species comparisons between *Parus major* and *Turdus merula* for the same tick species; shared letters indicate no statistically significant difference;
In column 'D': P-values for tests that compare the overall values (night and day combined) between nymph and larva;
(* N°): number of ticks that were isolated from birds, because no detachment took place; Columns 'Morning' and 'Evening': a,b,c describe tick species comparisons (order *I. ricinus, I. frontalis I. arboricola*); shared letters indicate no statistically significant difference.

3.1.4. Nymphal detachment in relation to moment of exposure

No effects of moment of exposure were observed, except for *I. frontalis* nymphs fed on blackbirds, which tended to detach more often during the night when birds were exposed in the evening ($\Delta_{(dusk\text{-}dawn)} = 14.7 \pm 7.2\%$ per bird).

3.2. Feeding duration

All *I. ricinus* larvae and nymphs detached within 6 days after infestation (see Fig. 2B). This contrasts with *I. arboricola* (data from great tits only) from which 10 nymphs and 42 larvae had to be isolated – still being attached on the bird - after an observation period of 20 days. All of them showed visual signs of successful engorgement (pers. obs.). Therefore, in larvae the estimated hazard for *I. arboricola* to detach was lower than *I. frontalis* (Hazard ratio (HR) $_{(I.a\ -\ I.f.)} = 0.12$; 95%-Confidence interval (CI): 0.07-0.23; $\chi^2 = 41.47$; df = 1; P < 0.0001) and *I. ricinus* (HR $_{(I.a\ -\ I.r.)} = 0.10$; CI: 0.06-0.16; $\chi^2 = 97.24$; df = 1; P < 0.0001) at any given time point. In nymphs, the estimated hazard to detach in *I. arboricola* was lower than in the other ticks (HR $_{(I.a\ -\ I.f.\ and\ I.a\ -\ I.r.)} = 0.47$ and 0.28; CI: 0.15-1.47 and 0.13-0.61; $\chi^2 = 1.69$ and 10.13; df = 1; all P's < 0.03).

3.2.1. Larval feeding duration in relation to host species and moment of exposure

Ixodes frontalis and *I. ricinus* detached on average sooner from blackbirds than from great tits (HR $_{(P.m.\text{-}T.m.)} = 0.48$; CI: 0.30-0.76; $\chi^2 = 9.70$; df = 1; P = 0.002). *Ixodes frontalis* and *I. arboricola* placed on great tits at dusk stayed significantly shorter on the birds than those placed at dawn ($\chi^2 > 4.91$; df = 1; P < 0.007). In contrast, *I. frontalis* ticks placed on blackbirds at dusk tended to detach later (HR $_{(dusk\text{-}dawn)} = 0.67$; CI: 0.34-1.32; $\chi^2 = 1.36$; df = 1; P = 0.014). In both great tits and blackbirds, *I. ricinus* placed at dusk remained attached on average longer (HR $_{(dusk\text{-}dawn)} = 0.48$; CI: 0.30-0.76; $\chi^2 = 9.70$; df = 1; P = 0.002).

3.2.2. Nymphal feeding duration in relation to host species and moment of exposure

As observed in larvae, nymphs detached sooner from blackbirds than from great tits (HR $_{(P.m.\text{-}T.m.)} = 0.13$; CI: 0.09-0.19; $\chi^2 = 133.14$; df = 1; P < 0.0001). Furthermore, they detached sooner from blackbirds than larvae, both in *I. frontalis* and *I. ricinus* (HR $_{(larva\text{-}nymph)} = 0.14$; CI: 0.11-0.18; $\chi^2 = 268.15$; df = 1; P < 0.0001); a difference not observed in great tits. In great tits, we found no evidence that 'moment of exposure' has an effect on feeding duration (all P's ≥ 0.05). However, in blackbirds, *I. frontalis* nymphs placed on the birds at dusk detached slightly sooner (HR $_{(dusk\text{-}dawn)} = 2.06$; CI: 1.12-3.79; $\chi^2 = 5.40$; df = 1; P = 0.012).

3.3. Feeding success

More *I. frontalis* than *I. ricinus* were recovered after feeding in great tits (logit $_{(I.r\ -\ I.f.)} = 0.43 \pm 0.12$; $\chi^2 = 7.33$; df = 1; P= 0.007) but no such

difference was found in blackbirds. No statistically significant effects were found of 'moment of exposure' in any of the tick - bird combinations, except for *I. arboricola* larvae: the ones placed at dusk tended ($\chi^2 = 2.93$; df = 1; P= 0.086) to be more successful than the ones placed at dawn (logit $_{(dusk - dawn)} = 0.35 \pm 0.17$).

4. Discussion

The life history of the bird-specialist tick *I. frontalis* has been poorly studied, despite the tick's proven vector capacity for human pathogens (Heylen et al., 2017a) and at least occasional records of biting humans (Gilot et al., 1997). Our study focused on the behavioural decisions immature developmental stages make at the end of feeding on diurnally active birds, which are its main host types (Norte et al., 2012). Given the tick's low intrinsic mobility, timing of detachment and duration of feeding should be directly linked to its off-host habitat requirements and its dispersal capability. As regards the detachments, two scenarios are possible: when ticks detach during the night, fed ticks end up underneath the sleeping bird, either inside the cavity or at the base of brushes and trees. This strategy would increase survival and host localization, given the roosting-site fidelity observed in many birds. In the other scenario, when ticks detach during the day, they are scattered in the open, but still in the vicinity of bushes, shrubbery and trees (depending on the songbird's habitat use). This strategy would increase dispersal, which can be advantageous in escaping density-dependent mortality in future infestation attempts, and in avoiding inbreeding.

We observed contrasts in the propensity to detach during the night between tick species, tick developmental stages and host types. *Ixodes ricinus* is exophilic (i.e. parasitic lifestyle characterized by questing for passing hosts), whereas *I. arboricola* is endophilic (i.e. parasitic lifestyle characterized by living in or near the nest or burrow of the hosts). As expected (Balashov, 1972), the exophilic *I. ricinus* -which feeds on a variety of vertebrate host types that live close to the forest floor (Gray, 1998) - detached mostly diurnally. In contrast, the endophilic

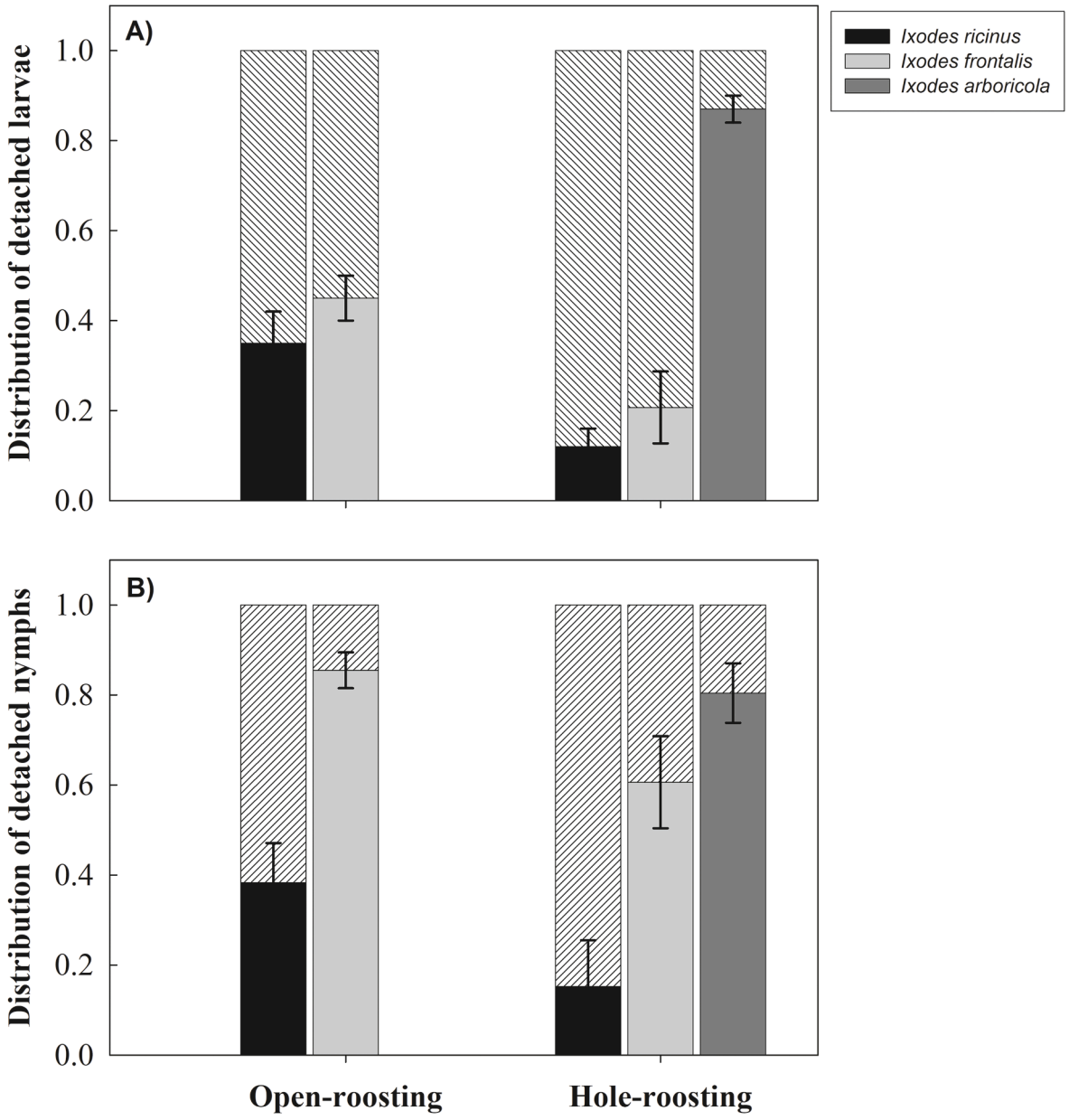

Fig. 1. Proportion of *Ixodes ricinus* (black fill color), *I. frontalis* (light grey fill color), *I. arboricola* (dark grey fill color) larvae (A) and nymphs (B) that have detached during the night. Open-roosting and hole-roosting birds were represented by blackbirds (*Turdus merula*) and great tits (*Parus major*), respectively. No data have been obtained for the combination *I. arboricola* - open-roosting bird.

I. arboricola detached mostly nocturnally, increasing the chances for a next encounter with a hole-roosting bird in its preferred habitat. Overall, we found detachments of *I. frontalis* larvae to be comparable with *I. ricinus*, but those of nymphs to be comparable with *I. arboricola* (Fig. 1). Therefore, the data suggest that the reproductive adult stage moulted from the engorged nymph tends to find its host near the birds' roosting site (cf. *I. arboricola*). Infesting a roosting bird has two main advantages: the tick avoids intensive grooming behaviour, because a sleeping bird shows less of this activity. Especially adult female ticks are very large (Heylen et al., 2014a), and therefore prone to be detected and groomed away by an alert bird. Secondly, birds often return to the same locations or habitat type where they roosted before (i.e. roostsite fidelity). *Ixodes frontalis* ticks detached less frequently at night from the hole-roosting great tit. We hypothesize that ticks link the physiological characteristics of the hole-roosting bird to its habitat (i.e. cavity) which *I. frontalis* does not prefer, due to abiotic conditions and/or the lack of suitable host types. Indications that point in this same direction are the following: (1) *Ixodes frontalis*' host range extends beyond hole-breeding birds: it actually includes a broad diversity of bird taxa, predominantly open-roosting birds. In a field study (Norte et al., 2012), ground-dwelling open-roosting birds (in particular blackbirds and European robins *Erithacus rubecula*) were 3-5 times more often infested by *I. frontalis*. (2) in our long-term Belgian monitoring study, *I. frontalis* was rarely found in bird nests (> 600 nest boxes checked) although the ticks have been observed on hole-breeding birds. (3) In an *ad hoc* infestation in the wild, we found that *I. frontalis* larvae that fed on great tit nestlings crawled outside the nestbox opening, after which they let themselves drop on forest litter (pers. obs. D. Heylen).

Most plausible substrates on which unfed stages of *I. frontalis* encounter their hosts are the (vegetation or foliage underneath) branches where birds roost and forage. Flagging of the vegetation and foliage in urban gardens in Germany (Agoulon et al., 2019; Drehmann

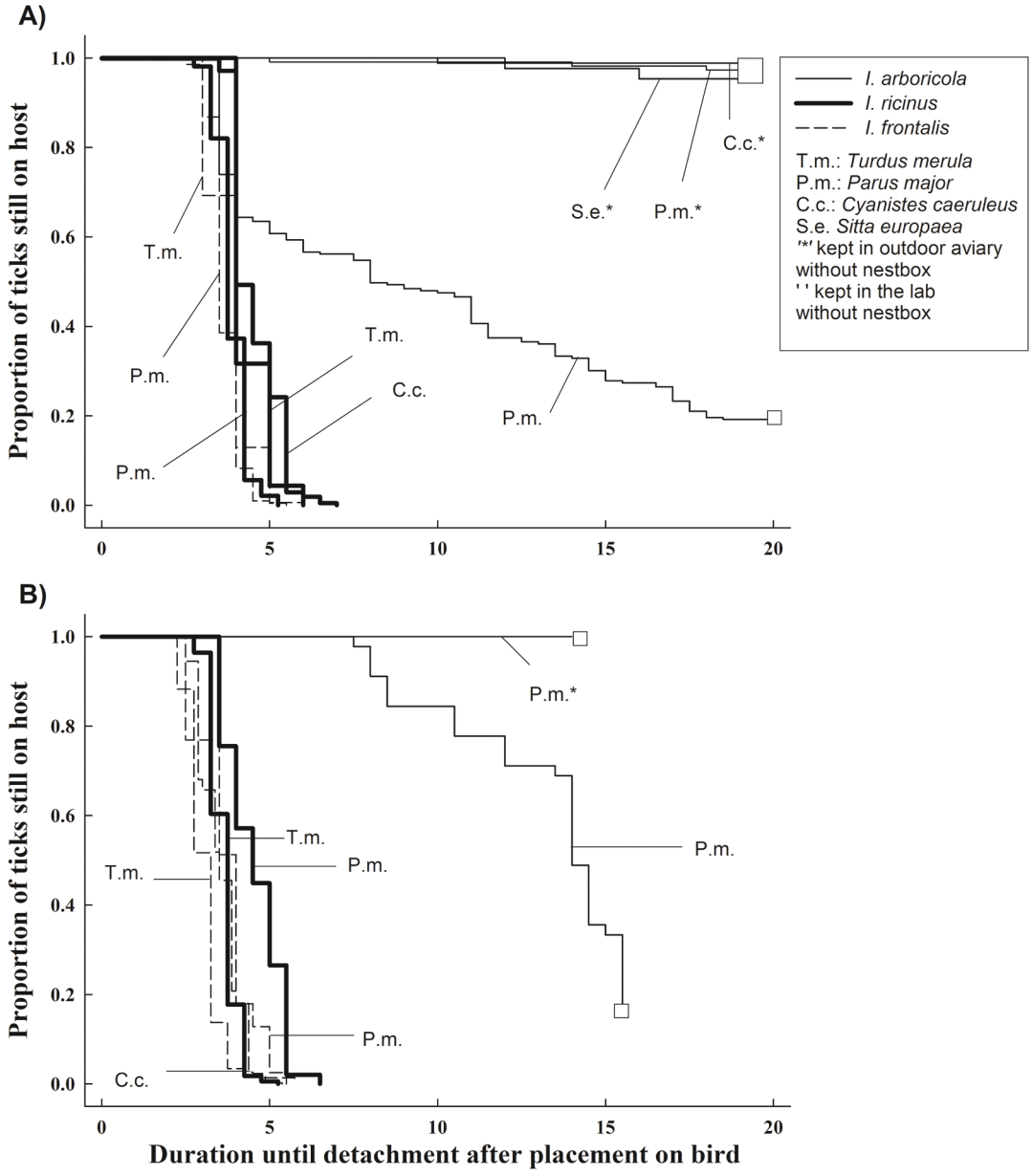

Fig. 2. Kaplan-Meier curves of the detachment from the birds in *Ixodes arboricola* (thin solid lines), *I. ricinus* (bold solid lines) and *I. frontalis* (dash lines) larvae (A) and nymphs (B). Open boxes represent censored ticks (i.e. ticks that were removed as they did not detach). Different bird species and conditions in which birds were kept are indicated in the figure's legend (see Table 1 and 'Materials and Methods' section for further details). *Turdus merula* is an open-roosting bird, all others are hole-roosting.

et al., 2019) and the foliage underneath Bamboo bushes in France (a non-native plant species where birds roost and find cover) resulted in the collection of the three parasitic stages of *I. frontalis* (Agoulon et al., 2019; Plantard et al., 2021). In the above-mentioned studies, the tick shows somewhat similar questing behaviour as the exophilic *I. ricinus* that was found in the same environments. However, low numbers of *I. frontalis* adults were collected, which indicates (1) adults did not show the questing behaviour of the immature developmental stages at the moment of collections (i.e. during the day) and/or (2) they have to be found elsewhere – possibly on branches comprising the roosting sites of open-roosting birds such as blackbirds. The precise *I. frontalis* micro-habitats within the vegetation still require further elucidation.

Feeding durations of *I. frontalis* were similar to *I. ricinus*: within a week all ticks detached. This contrasts with *I. arboricola*, which shows the capacity to stay for very long periods of time on the host - in the absence of a cavity. It seems likely this is a strategy to increase the chance to end up in its preferred tree-hole habitat (White et al., 2012). Furthermore, the chance that birds change roosting or foraging locations increases with time, which increases the dispersal possibilities in this habitat-restricted tick species. But in general, expediting engorgement and detachment are assumed to be beneficial for the tick individual, as the shorter time period the tick needs to be on the host, the less likely it will be groomed away. Hence, the shorter feeding durations of *I. frontalis* immature developmental stages are likely to be to the advantage of the tick on the host.

Our experiments show that a life-history characteristic, the moment of leaving the host, differs among congeneric parasites, but also varies between host species. Although the three tick species co-occur in the same macro-habitat, they have different ecologies, micro-habitat requirements and different host specificities; we suggest that those differences are partly displayed in the detachment rhythms. Our study indicates that habitat requirements and host suitability drive the evolution of the observed tick responses, here showing phenotypic plasticity. The question remains how these contrasts have evolved: as a consequence of host dependence (i.e. host-driven) or as a consequence of the off-host habitat dependence. Also, the exact cues used by the ticks for leaving the host (e.g. physiological change in host, or external light/dark cycles) are poorly understood. Further research may reveal to what extent our observations may effectively contribute to important ecological features in transmission cycles of the ticks and their pathogens: virulence, host specificity, dispersal, and competition.

CRediT authorship contribution statement

D.J.A. Heylen: Writing – original draft, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization. **A.R. Van Oosten:** Writing – original draft, Data curation, Methodology, Investigation. **G. Fracasso:** Writing – original draft. **E. Matthysen:** Writing – original draft, Project administration.

Acknowledgements

DH is funded by the Marie Sklodowska-Curie Actions (EU-Horizon 2020, Individual Global Fellowship, project no 799609). We are grateful to Joris Elst and Frans Fierens for their technical assistance, and Annelien Vancauwenberghe for constructive comments on previous versions of the manuscript.

References

Agoulon, A., Hoch, T., Heylen, D., Chalvet-Monfray, K., Plantard, O., 2019. Unravelling the phenology of *Ixodes frontalis*, a common but understudied tick species in Europe. Tick. Tick. Borne. Dis. 10, 505–512. https://doi.org/10.1016/j.ttbdis.2018.12.009.

Balashov, Y.S., 1972. Bloodsucking Ticks (Ixodidea) - Vectors of Diseases of Man and Animals, 8. Miscellaneous Publications of the Entomological Society of America, pp. 159–376.

Bona, M., Stanko, M., 2013. First records of the tick *Ixodes frontalis* (Panzer, 1795) (Acari, Ixodidae) in Slovakia. Tick. Tick. Borne. Dis. 4, 478–481. https://doi.org/10.1016/j.ttbdis.2013.06.002.

Chastel, C., Chandler, L., Le Goff, F., Chastel, O., Tesh, R., Shope, R., 1999. Chize virus, a new phlebovirus isolated in France from *Ixodes* (*Trichotoixodes*) *frontalis*. Acta Virol. 43, 279–283.

Chen, H., Smith, G.J., Zhang, S.Y., Qin, K., Wang, J., Li, K.S., Webster, R.G., Peiris, J.S., Guan, Y., 2005. Avian flu: H5N1 virus outbreak in migratory waterfowl. Nature 436, 191–192. https://doi.org/10.1038/nature03974.

Dick, C.W., Patterson, B.D., 2007. Against all odds: Explaining high host specificity in dispersal-prone parasites. Int. J. Parasitol. 37, 871–876. https://doi.org/10.1016/j.ijpara.2007.02.004.

Doby, J.M., 1998. Contribution à la connaissance de la biologie de *Ixodes* (*Trichotoixodes*) *pari* Leach (= *I. frontalis* (Panzer)) (Acari: Ixodidae), tique spécifique des oiseaux. Acarologia 39, 315–325.

Drehmann, M., Chitimia-Dobler, L., Lindau, A., Frank, A., Mai, S., Fachet, K., Hauck, D., Knoll, S., Strube, C., Luhken, R., Fischer, D., Ziegler, L., Mackenstedt, U., 2019. *Ixodes frontalis*: a neglected but ubiquitous tick species in Germany. Exp. Appl. Acarol. 78, 79–91. https://doi.org/10.1007/s10493-019-00375-3.

Estrada-Peña, A., Oteo, J.A., Estrada-Peña, R., Gortazar, C., Osacar, J.J., Moreno, J.A., Castella, J., 1995. *Borrelia burgdorferi* sensu lato in ticks (Acari: Ixodidae) from two different foci in Spain. Exp. Appl. Acarol. 19, 173–180. https://doi.org/10.1007/BF00046289.

Fracasso, G., Matthysen, E., Dhondt, A., Heylen, D., 2019. Experimental study of micro-habitat selection in ixodid ticks feeding on avian hosts. Int. J. Parasitol. 49, 1005–1014.

Gilot, B., Beaucournu, J.C., Chastel, C., 1997. Fixing on man of *Ixodes* (*Trichotoixodes*) *frontalis* (Panzer, 1795) collected with the flagging method. Parasite 4, 197–199. https://doi.org/10.1051/parasite/1997042197.

Ginsberg, H.S., Buckley, P.A., Balmforth, M.G., Zhioua, E., Mitra, S., Buckley, F.G., 2005. Reservoir competence of native north American birds for the Lyme disease spirochete, *Borrelia burgdorferi*. J. Med. Entomol. 42, 445–449. https://doi.org/10.1603/0022-2585(2005)042[0445:Rconna]2.0.Co;2 https://doi.org/doi/.

Gray, J.S., 1998. The ecology of ticks transmitting Lyme borreliosis. Exp. Appl. Acarol. 22, 249–258. https://doi.org/10.1023/A:1006070416135 https://doi.org/doi/.

Heylen, D., De Coninck, E., Jansen, F., Madder, M., 2014a. Differential diagnosis of three common Ixodes spp. ticks infesting songbirds of Western Europe: *Ixodes arboricola, I. frontalis* and *I. ricinus*. Tick. Tick. Borne. Dis. 5, 693–700. https://doi.org/10.1016/j.ttbdis.2014.05.006.

Heylen, D., Krawczyk, A., Lopes de Carvalho, I., Nuncio, M.S., Sprong, H., Norte, A.C., 2017a. Bridging of cryptic *Borrelia* cycles in European songbirds. Environ. Microbiol. 19, 1857–1867. https://doi.org/10.1111/1462-2920.13685.

Heylen, D., Matthysen, E., Fonville, M., Sprong, H., 2014b. Songbirds as general transmitters but selective amplifiers of *Borrelia burgdorferi* sensu lato genotypes in *Ixodes rinicus* ticks. Environ. Microbiol. 16, 2859–2868. https://doi.org/10.1111/1462-2920.12304.

Heylen, D., Tijsse, E., Fonville, M., Matthysen, E., Sprong, H., 2013. Transmission dynamics of *Borrelia burgdorferi* s.l. in a bird tick community. Environ. Microbiol. 15, 663–673. https://doi.org/10.1111/1462-2920.12059.

Heylen, D.J., Matthysen, E., 2010. Contrasting detachment strategies in two congeneric ticks (Ixodidae) parasitizing the same songbird. Parasitology 137, 661–667. https://doi.org/10.1017/S0031182009991582.

Heylen, D.J., Sprong, H., Krawczyk, A., Van Houtte, N., Genne, D., Gomez-Chamorro, A., van Oers, K., Voordouw, M.J., 2017b. Inefficient co-feeding transmission of *Borrelia afzelii* in two common European songbirds. Sci. Rep. 7, 39596. https://doi.org/10.1038/srep39596.

Heylen, D.J.A., Matthysen, E., 2008. Effect of tick parasitism on the health status of a passerine bird. Funct. Ecol. 22, 1099–1107. https://doi.org/10.1111/j.1365-2435.2008.01463.x.

Hillyard, P.D., 1996. Ticks of North-West Europe. Backhuys Publishers, London.

Hubalek, Z., 2004. An annotated checklist of pathogenic microorganisms associated with migratory birds. J. Wildl. Dis. 40, 639–659. https://doi.org/10.7589/0090-3558-40.4.639.

Hudde, H., Walter, G., 1988. Verbreitung und Wirtswahl der Vogelzecke *Ixodes arboricola* (Ixodoidea, Ixodidae) in der Bundesrepublik Deutschland. Vogelwarte 34, 201–207.

Lanciotti, R.S., Roehrig, J.T., Deubel, V., Smith, J., Parker, M., Steele, K., Crise, B., Volpe, K.E., Crabtree, M.B., Scherret, J.H., Hall, R.A., MacKenzie, J.S., Cropp, C.B., Panigrahy, B., Ostlund, E., Schmitt, B., Malkinson, M., Banet, C., Weissman, J., Komar, N., Savage, H.M., Stone, W., McNamara, T., Gubler, D.J., 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 286, 2333–2337. https://doi.org/10.1126/science.286.5448.2333.

Literak, I., Kocianova, E., Dusbabek, F., Martinu, J., Podzemny, P., Sychra, O., 2007. Winter infestation of wild birds by ticks and chiggers (Acari: Ixodidae, Trombiculidae) in the Czech Republic. Parasitol. Res. 101, 1709–1711. https://doi.org/10.1007/s00436-007-0702-9.

McCoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2003. Host-dependent genetic structure of parasite populations: differential dispersal of seabird tick host races. Evolution 57, 288–296. https://doi.org/10.1111/j.0014-3820.2003.tb00263.x.

Movila, A., Alekseev, A.N., Dubinina, H.V., Toderas, I., 2013. Detection of tick-borne pathogens in ticks from migratory birds in the Baltic region of Russia. Med. Vet. Entomol. 27, 113–117. https://doi.org/10.1111/j.1365-2915.2012.01037.x.

Norte, A.C., de Carvalho, I.L., Ramos, J.A., Goncalves, M., Gern, L., Nuncio, M.S., 2012. Diversity and seasonal patterns of ticks parasitizing wild birds in western Portugal. Exp. Appl. Acarol. 58, 327–339. https://doi.org/10.1007/s10493-012-9583-4.

Ogden, N.H., Lindsay, L.R., Hanincova, K., Barker, I.K., Bigras-Poulin, M., Charron, D.F., Heagy, A., Francis, C.M., O'Callaghan, C.J., Schwartz, I., Thompson, R.A., 2008. Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. Appl. Environ. Microbiol. 74, 1780–1790. https://doi.org/10.1128/AEM.01982-07.

Plantard, O., Hoch, T., Daveu, R., Rispe, C., Stachurski, F., Boue, F., Poux, V., Cebe, N., Verheyden, H., Rene-Martellet, M., Chalvet-Monfray, K., Cafiso, A., Olivieri, E., Moutailler, S., Pollet, T., Agoulon, A., 2021. Where to find questing *Ixodes frontalis* ticks? Under bamboo bushes! Tick. Tick. Borne. Dis. 12, 101625 https://doi.org/10.1016/j.ttbdis.2020.101625, 101625.

Poulin, R., 2007. Evolutionary ecology of parasites, 2 ed. Princeton university press, Princeton.

Price, P.W., 1980. Evolutionary biology of parasites. Princeton University Press, Princeton.

Rubel, F., Brugger, K., Chitimia-Dobler, L., Dautel, H., Meyer-Kayser, E., Kahl, O., 2021. Atlas of ticks (Acari: Argasidae, Ixodidae) in Germany. Exp. Appl. Acarol. 84, 183–214. https://doi.org/10.1007/s10493-021-00619-1.

Schorn, S., Schol, H., Pfister, K., Silaghi, C., 2011. First record of *Ixodes frontalis* collected by flagging in Germany. Tick. Tick. Borne. Dis. 2, 228–230. https://doi.org/10.1016/j.ttbdis.2011.07.003.

Shu, Y., Klein, J.P., 1999. A SAS macro for the positive stable frailty model. In: Proceedings of the Statistical Computing Section. Baltimore, Maryland. American Statistical Association, pp. 47–52.

White, J., Heylen, D.J., Matthysen, E., 2012. Adaptive timing of detachment in a tick parasitizing hole-nesting birds. Parasitology 139, 264–270. https://doi.org/10.1017/S0031182011001806.